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Soldier morphogenesis in a nasute termite: discovery of a disc-like structure forming a soldier nasus

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Nasute termites belonging to the subfamily Nasutitermitinae, have a soldier caste that possesses a frontal projection (nasus) on the head, from which defensive substances are secreted. In the course of caste differentiation of the processional nasute termite *Hospitalitermes medioflavus*, the most dynamic morphogenesis occurs in the stage of moulting from male minor worker to presoldier (the stage preceding the soldier stage). We examined the presumptive nasus epithelium in minor workers and determined that the nasus develops rapidly just prior to the moulting to presoldiers. The rapid growth is associated with two folding layers of cuticle and epithelium, which we termed the soldier-nasus disc, and resembles the imaginal discs found in holometabolous insects.

Keywords: caste differentiation; disc; male individuals; nasus; soldier; termite

1. INTRODUCTION

The soldier caste of termites is morphologically and functionally distinct from all other castes in a termite colony (Weesner 1969). Termite soldiers attack predators with large and strong mandibles and/or use defensive chemical substances mainly secreted from a frontal gland (Prestwich 1983). However, the nasute termites belonging to the subfamily Nasutitermitinae, which is the most apical group in recent phylogenetic reconstructions of higher termites (family Termitidae) (Miura *et al.* 1998*a*), have soldiers with a conspicuous projection at the head, the 'nasus'. Soldiers secrete viscous substances from the nasus that repel predators. This distinctive soldier morphology arises during post-embryonic development with dynamic morphological changes occurring during development of the presoldier.

In this study, the formation of the nasus structure in the soldiers of the nasute termites *Hospitalitermes medioflavus* was investigated. The soldiers develop from minor workers via a presoldier stage, with two moulting events. In this and related species only males differentiate into soldiers (Noirot 1955, 1969; Roisin 1992, 1996; Miura *et al.* 1998*b*), although in other groups both sexes can develop into soldiers (Noirot 1955, 1969).

2. MATERIAL AND METHODS

(a) *Study site and specimen collection*

Specimens of *H. medioflavus* were collected in Bukit Soeharto Protection Forest, East Kalimantan, Indonesia. Five nests of the focal species were opened and a number of individuals were collected randomly. Specimens were fixed in FAA solution (formaldehyde:ethanol:acetic acid, 6:16:1) for about 24 h in preparation for histological observations. After fixation, samples were preserved in 70% ethanol (EtOH). For scanning electron micrographs, individuals were preserved directly in 70% EtOH solution. Individuals were discriminated into each caste, based on the previously investigated caste developmental pathways of this species (Miura & Matsumoto 1995; Miura *et al.* 1998*a*).

(b) *Scanning electron micrographs*

Scanning electron microscopy was used to investigate the formation of the nasus structure in the stage prior to the presoldier (i.e. minor worker). Samples fixed in FAA and then preserved in 70% EtOH were dehydrated by transfer into increasing concentrations of ethanol (up to 100%), then into acetone, and finally into hexamethyldisilazane. After being air-dried, samples were coated with gold by using an ion coater (Eiko IB-3, Eiko Engineering, Ibataki, Japan). Scanning electron micrographs were taken with a Hitachi S-405 (Hitachi Instruments, Tokyo, Japan).

(c) *Histological observations*

To observe the nasus formation histologically, paraffin sections were made and stained with haematoxylin and eosin. The whole body or head of a termite individual was dehydrated in increasing concentrations of the EtOH, then transferred into xylene, and finally embedded into paraffin. Successive sections (5 µm in thickness), were mounted on glass slides coated with egg-white glycerin. Paraffin was removed from sections with xylene. Sections were stained by transferring into decreasing concentrations of EtOH, and then into haematoxylin solution (0.1% haematoxylin, 0.02% $NaIO_3$, 5% $AlK(SO_4)_2$, 0.1% citric acid, 5% trichloroacetaldehyde). Sections were de-stained in running water, then transferred into 0.5% eosin solution, and then de-stained again. After staining, slides were dehydrated and enclosed with Canada balsam under cover-glasses.

3. RESULTS

In *H. medioflavus*, it is already known that the soldiers differentiate from minor workers via a presoldier stage (Miura *et al.* 1998*b*; figures 1 and 2). Therefore, minor workers were selected for study from the specimens fixed

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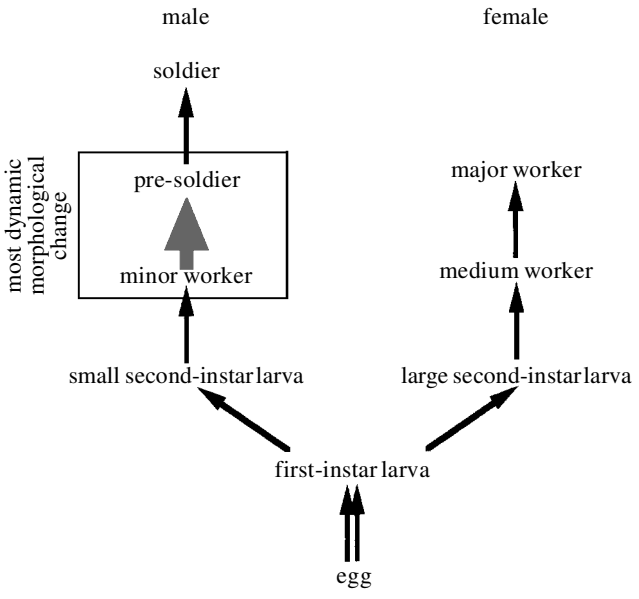

Figure 1. Scheme of caste developmental pathways in *H. medioflavus* (based on Miura *et al.* (1998*b*)). The most dynamic morphogenesis is seen when the moulting from minor worker to presoldier occurs in male neuters.

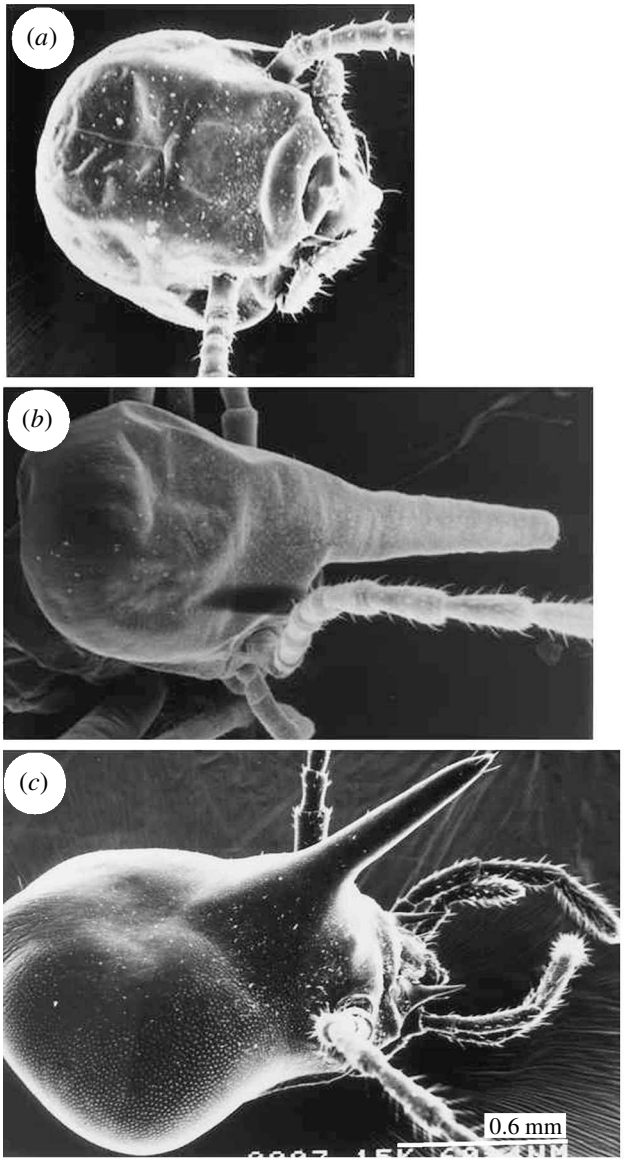

Figure 2. Scanning electron micrographs showing the morphology of the heads of (*a*) minor worker, (*b*) presoldier and (*c*) soldier. The nasus structure appears through the single moulting from minor worker to presoldier.

in FAA, based on the size of the head capsule (Miura & Matsumoto 1995). Minor workers approaching the next moult could be identified by the fatty and yellowish-white colour of their abdomens. The head capsule of the individuals was dissected, revealing the developing structure of the nasus, which exhibited concentric circles of tissue (figure 3*a*,*b*). The centre of the concentric circles started to elongate at the same time the cuticle of the minor worker head split, and the nasus structure extended outward (figure 3*c*). For comparison with other moulting individuals, the head of the moulting medium worker was also examined. In this case, the epithelium did not have concentric circles of folded tissue, but instead had the characters of the worker head showing a groove along the midline (figure 3*d*).

Histological observations of the heads in various stages of male neuter castes (figure 4) showed that the structure forming the nasus was similar to the imaginal disc which is seen in larvae of holometabolous insects (e.g. Gilbert 1997, p. 749). To determine the starting time in the formation of the disc-like structure, second instar of male larvae and functional minor workers were also examined. In the heads of both, no disc-like structure was found (figure 4*a*,*b*), which implies that the disc was formed just prior to the moult into the presoldier (figure 4*c*,*d*). The sections showed that the nasus disc had a bilayer structure composed of an outer cuticle layer and an inner epithelial layer. Both layers were folded and the epithelial cells were extended along their basal–apical axis (figure 4*d*). Then after moulting, the folded layers expanded to elongate into the nasus structure of presoldiers (figure 4*e*).

4. DISCUSSION

Termite soldiers develop through two moulting events; the first one involves moulting into a presoldier, and the second into a soldier. Presoldiers are an immature precursor caste, with an unpigmented cuticle and a white appearance, although they possess the basic morphological features of soldiers. Therefore, the most dynamic morphogenetic changes of the soldier caste occur prior to and during the moult into presoldiers. In hemimetabolous insects, morphs typically develop gradually during post-embryonic nymphal development. Only at the moult into the imago does morphogenesis of wings, eyes and other organs occur dynamically. Although termites are hemimetabolous insects as well, the morphogenesis of the soldier can be regarded as a distinctive moulting event in the life cycle of this insect.

This study revealed that an imaginal disc-like structure develops under the cuticle of the head of minor workers which are approaching the moult (figure 3). At this time, the gut contents of moulting individuals were excreted. This stage is, therefore, similar to the prepupal

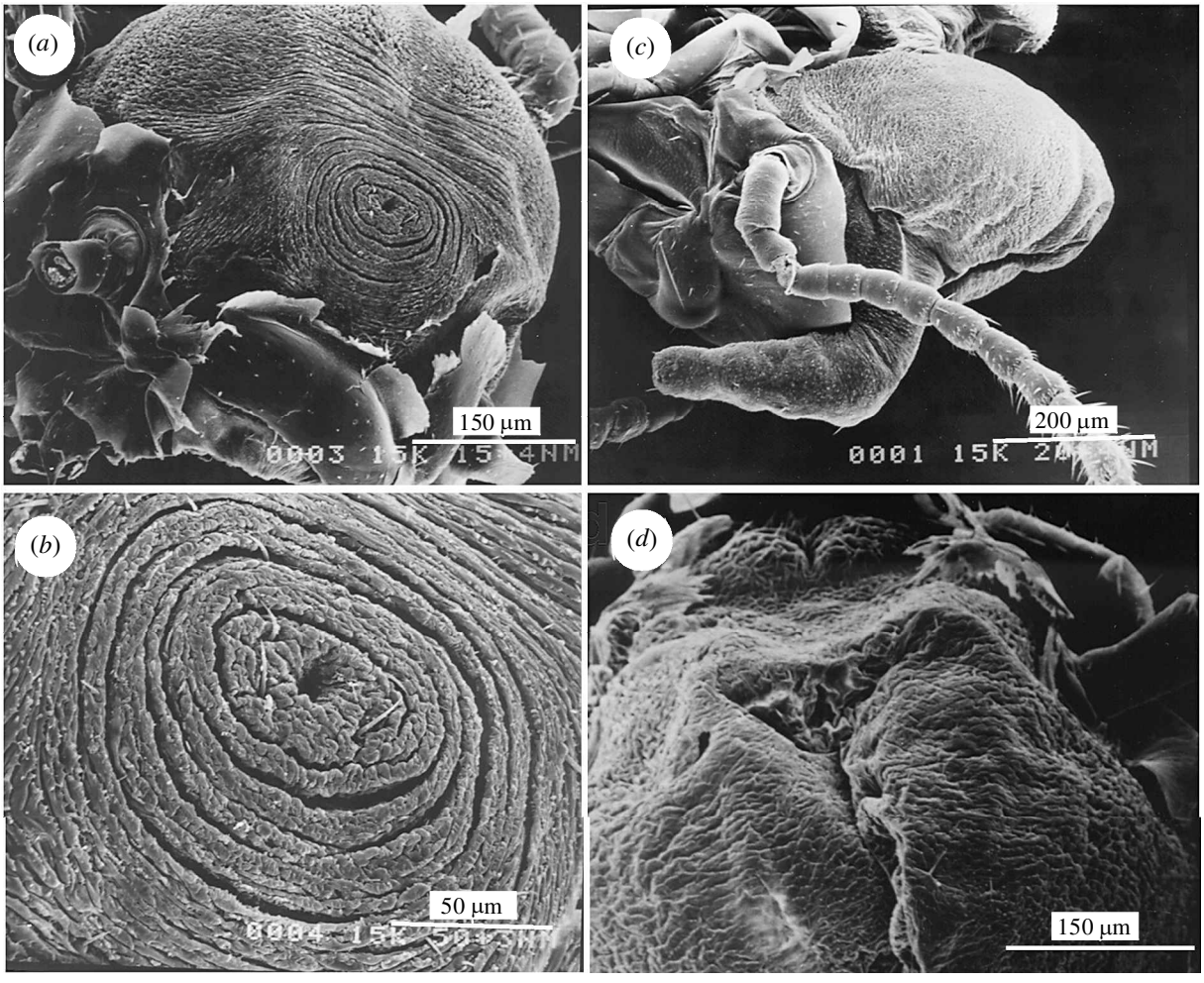

Figure 3. Scanning electron micrographs of the soldier-nasus disc, which is constructed under the cuticle of the head of the minor worker (*a*,*b*), and the centre of the disc elongates towards the distal direction when the moulting into presoldiers occurs (*c*). Under the cuticle of female medium workers, the disc-like structure does not appear even when the moulting events occur (*d*).

stage of holometabolous insects, when metamorphosis occurs. In the imaginal discs of holometabolous insects, cells are initially compressed, and are released to elongate the tissue after a pulse of ecdysone triggers metamorphosis (Condic *et al.* 1990). A similar compression and then release of cells is observed in the nasus disc.

Considering the fact that ecdysteroids initiate the elongation of the imaginal disc of *Drosophila* (Gilbert 1997, p. 749), some hormones associating with moulting may also initiate the formation and elongation of the soldier-nasus disc. The differentiation to presoldiers is induced by juvenile hormone (JH) and/or its analogues in some termite species (e.g. Howard & Haverty 1979; Nijhout & Wheeler 1982), therefore JH may be related to the formation of the disc. In our preliminary experiments on a lower termite, JH initiated the differentiation into presoldiers, whereas JH does not promote moulting but rather inhibits it after the onset of the differentiation (T. Miura, unpublished data). High levels of JH repress the metamorphic pulse in other insects (Nijhout 1994). This suggests that JH may be needed for the formation of the nasus disc but other hormone(s) such as ecdysone may initiate its elongation.

Thus far, imaginal disc-like structures have never been reported in any hemimetabolous insects, therefore the discovery of the soldier-nasus disc in this study may give us deeper insights into the development not only of social insects but of insects in general. Parallel evolution of developmental strategies for dramatic morphological change may have occurred in the evolution of the soldier caste, and similar mechanisms of development may have evolved in the phylogenetically distant insects.

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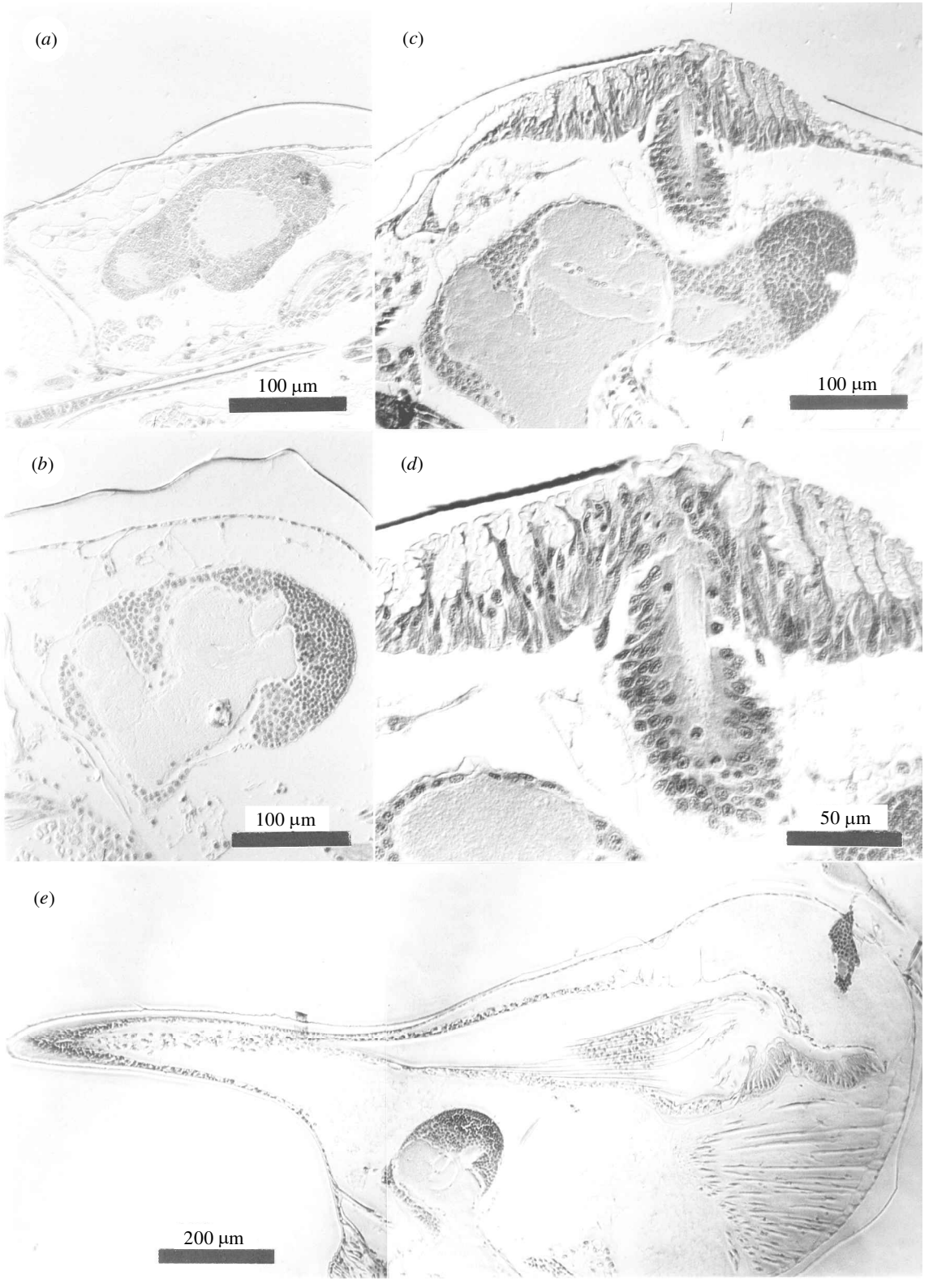

Figure 4. Cross-sections (longitudinal plane) of the heads in the male neuter castes in *H. medioflavus*; (*a*) second-instar larva, (*b*) functional minor worker, (*c*,*d*) moulting minor worker and its soldier-nasus disc, and (*e*) presoldier. The formation of the soldier-nasus disc, constructed with two layers of cuticle and epithelium, occurred just prior to the moulting into presoldiers. It is seen neither in the second-instar larva nor in the functional minor worker.

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